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Wirta, Helena K.

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Complementary molecular information changes our perception of food web structure

Helena K. Wirta[a,1], Paul D. N. Hebert[b], Riikka Kaartinen[a,2], Sean W. Prosser[b], Gergely Várkonyi[c], and Tomas Roslin[a]

[a]Spatial Foodweb Ecology Group, Department of Agricultural Sciences, University of Helsinki, FI-00014, Helsinki, Finland; [b]Biodiversity Institute of Ontario, University of Guelph, Guelph, ON, Canada N1G 2W1; and [c]Natural Environment Centre, Finnish Environment Institute, FI-88900, Kuhmo, Finland

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How networks of ecological interactions are structured has a major impact on their functioning. However, accurately resolving both the nodes of the webs and the links between them is fraught with difficulties. We ask whether the new resolution conferred by molecular information changes perceptions of network structure. To probe a network of antagonistic interactions in the High Arctic, we use two complementary sources of molecular data: parasitoid DNA sequenced from the tissues of their hosts and host DNA sequenced from the gut of adult parasitoids. The information added by molecular analysis radically changes the properties of interaction structure. Overall, three times as many interaction types were revealed by combining molecular information from parasitoids and hosts with rearing data, versus rearing data alone. At the species level, our results alter the perceived host specificity of parasitoids, the parasitoid load of host species, and the web-wide role of predators with a cryptic lifestyle. As the northernmost network of host–parasitoid interactions quantified, our data point exerts high leverage on global comparisons of food web structure. However, how we view its structure will depend on what information we use: compared with variation among networks quantified at other sites, the properties of our web vary as much or much more depending on the techniques used to reconstruct it. We thus urge ecologists to combine multiple pieces of evidence in assessing the structure of interaction webs, and suggest that current perceptions of interaction structure may be strongly affected by the methods used to construct them.

trophic interaction | Hymenoptera | Lepidoptera | DNA barcode

How networks of ecological interactions are structured has major implications on how they function (1), how they react to external stressors (2, 3) and how they return to their original state after disturbance (4, 5). Quantitative descriptions of who interacts with whom (e.g., refs. 6 and 7) may then be used to formulate testable hypotheses for basic questions concerning, for example, the fundamental strength of indirect interactions (8, 9), or how a community will respond to invasive species, habitat modification, or climate change (e.g., refs. 10–14). In applied biology, quantifications of interaction structure have increasingly been used to examine the success of, for example, habitat restoration and management, the biological control of pests, and the conservation of biodiversity (6). Global comparisons of food web structure are underway, comparing the linking structure of local webs described from different latitudes with highly different species richness (15, 16).

To encapsulate the emergent properties of large networks of interactions, a set of quantitative descriptors has been proposed (e.g., refs. 2, and 17–19) and widely adopted (e.g., refs. 11 and 20–22). Commonly used metrics summarize the specialization of species at different trophic levels, such as the average number of species at a lower trophic level interacting with each species at the higher level (a web-wide feature called "generality"), the average number of species at the higher level using each species at the lower trophic level ("vulnerability"), or the average number of other species with which any species in the web will typically interact ("linkage density"). By scaling the number of links observed in a web to the potential maximum number (should all species at one level interact with all species at another), we obtain a convenient metric of how tightly trophic levels are woven together ("connectance"). "Nestedness" captures a further aspect of network-level organization, by describing the extent to which more specialized species interact with subsets of the species that generalist species interact with (22).

Importantly, these emergent descriptors of interaction structure have been found to affect how networks change through time, and how they respond to disturbances: for mutualistic interactions (such as plants versus pollinators or plants versus seed dispersers), higher connectance at the network level appears to promote stability. In antagonistic networks, the effects may be reversed with increased connectance actually decreasing stability (21, 23). Increased linkage density, on its part, may increase the persistence of structure of a food web (23). Finally, the relative nestedness of an interaction web has been shown to affect the stability of communities, with effects varying with the type of interaction examined (refs. 2, 21, 22, and 24, but see refs. 25 and 26).

Given the links between interaction structure, community functioning, and dynamics appearing to date, further mapping of function on structure emerges as a key priority for current research. However, understanding these links relies on the fundamental idea that descriptions of interaction structure are accurate and unbiased. In practice, however, reconstructing interaction structure is riddled with problems. How the architecture of interactions is perceived

Significance

Understanding the interaction structure of ecological assemblages is the basis for understanding how they vary in space and time. To reconstruct interactions in the High Arctic, we draw on three sources of information: two based on DNA sequence data and one on the rearing of parasitoids from their hosts. Overall, we show that a combination of all three techniques will not only provide high resolution for describing feeding associations among individual species, but also revamp our view of the overall structure of the target network. Thus, our findings suggest that combining several types of information will fundamentally change our impression of both how local interaction webs are structured, and how biotic interactions are patterned across the globe.

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[1]To whom correspondence should be addressed. E-mail: helena.wirta@helsinki.fi.

[2]Present address: Department of Ecology, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden.

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depends on the methods used and on taxonomic resolution (27, 28). If multiple taxa are inadvertently grouped within the nodes of a web, or if links are poorly resolved, we risk misunderstanding its composition (28, 29), and thus the functioning of the system.

In this context, molecular techniques are emerging as precision tools for reconstructing interaction structure. Such methods have recently been shown to offer high resolution in identifying the nodes of antagonistic interaction networks (30–32), and in pinpointing feeding associations among hosts and parasitoids (33–36), plants and herbivores (37, 38), and selected predators within larger food webs (39–44). Although resolving the modules of ecological interactions forms the basis for understanding larger interaction webs, no prior study has examined the extent to which these novel sources of information change our perception of emergent interaction structure.

In this paper, we examine how molecular information changes our view of structure across quantified webs of ecological interactions. As a model network, we target a species-poor food web in the High Arctic, offering maximum tractability in terms of species composition, minimal sampling biases, and maximal leverage in analyses of global patterns in interaction structure. The system consists of the dominant herbivores of the area, i.e., Lepidoptera, and a specific group of predators attacking them, the parasitoids, characterized by free-living adults and larvae developing on or within single prey individuals (called "host") (45), killing them in the process (46). Targeting the network of interactions between these two trophic layers, we test whether the new resolution conferred by molecular information will complement or reconfigure current perceptions of web architecture.

Specifically, we ask a series of successive questions regarding the impact of method on food web structure: (*i*) Do different sources of information reveal the same or different sets of specific links within our target web? (*ii*) What do different methods reveal with respect to the web-level impact of species with different life histories/characteristics? (*iii*) Building off the set of specific links and their relative importance emerging from different methods, how much do metrics of emergent food web structure change with the method applied? (*iv*) To benchmark the observed level of variation, how does variation among food webs described by different methods compare with variation among food webs described from different parts of the world?

Results

To enable the identification of all interacting species, DNA barcodes (47) were generated for each species of host Lepidoptera and their parasitoids (Hymenoptera: Ichneumonoidea; and Diptera: Tachinidae) occurring in the study area at Zackenberg Valley, Northeast Greenland (48). By designing PCR primers to selectively amplify the DNA of the host order but not the parasitoid, we successfully amplified, sequenced, and identified the larval host (to a species level) for 21.9% of 457 parasitoids caught as adults (*SI Text*, section 1). As the general approach was coined "MAPL" (molecular analysis of parasitoid linkages) by Rougerie et al. (36), we henceforth refer to it as "MAPL-AP" (where AP stands for "adult parasitoid" as the source tissue) in our study. By comparing the sequences recovered to our reference library [via the Barcode of Life Data (BOLD) Systems] (49), each sequence was unambiguously assigned to a host taxon, with a total of eight larval host species detected in the gut contents of the adult parasitoids (Table S1). The reverse approach of selectively amplifying and sequencing the DNA of parasitoids embedded in the tissue of host larvae (henceforth "MAPL-HL," where HL stands for "host larva" as the source tissue) yielded an identified parasitoid sequence from 20.9% of 1,195 hosts examined (*SI Text*, section 1 and Table S1). These sequences represented 12 of the 30 species known to parasitize Lepidoptera in the area. As we did not detect sequences with double peaks (suggestive of amplification of more than one species at a time), the incidence of multiple parasitoid individuals in the same host or of multiple host species in the same parasitoid must be low (*SI Text*, section 2).

Reconstructing the food web by molecular techniques revised our impression of overall network structure. In terms of link numbers, the resolution added by MAPL-AP and MAPL-HL nearly tripled the number of trophic links detected in the web compared with a description based on rearing alone (Fig. 1 and Table S2) (50). The majority of links resolved by rearing was also detected by molecular techniques (Fig. 1 and Table S2) for added estimates see *SI Text*, section 3 and Table S3). However, a remarkably different set of links was revealed by each molecular technique (Fig. 1). Regarding the strength of interactions, links detected exclusively by single methods were by no means weak in terms of frequency (i.e., numbers of individuals involved); rather, they show strong connections within the web (Fig. 1).

At the level of individual species within the webs, the application of MAPL-AP and MAPL-HL altered our perception of the host specificity of parasitoids and the parasitoid ranges of hosts: qualitative measures of generality (i.e., the average number of host taxa per parasitoid taxon) (17), vulnerability (the average number of parasitoids per host taxon), and linkage density (the diversity of interactions per species) all approximately tripled (Table S2).

Molecular information also changed our perception of the web-wide role of predators with a cryptic lifestyle. The parasitoids examined here are frequently partitioned into idiobionts and koinobionts (51), with idiobionts developing on immobilized and/or concealed hosts such as eggs or (pre)pupae, and koinobionts typically on more mobile hosts. Given this difference in the detectability of the hosts affected, interactions involving idiobionts are significantly harder to detect and quantify using host larvae as the source of information. As MAPL-AP specifically targets the free-living adult stage of the parasitoid, this technique allowed for the relatively easy detection of links between idiobionts and their hosts (Fig. 1*C*), thus resolving often-overlooked trophic connections with a significant impact on overall food web structure (Fig. 1*E*).

Finally and most prominently, networks reconstructed by each method not only varied in the numbers and identities of links, but also in their emergent structure (Figs. 1 and 2). This was reflected by pronounced differences in several key metrics (Table S2): notably, the connectance of the molecularly informed web was three times as high as that of the rearing-based web, whereas nestedness differed ninefold among webs reconstructed by different techniques.

To illustrate the degree to which the use of molecular information affects our impression of network structure, one may compare variation in the structure of our single target web reconstructed by different techniques with variation among five closely comparable webs from different parts of the world (as all reconstructed by traditional rearing) (10, 52–55). This comparison indicated that our rearing-based web for Zackenberg possessed the lowest generality of all six webs, whereas our molecularly informed web showed the highest generality (Fig. 2 and Table S2). For vulnerability, linkage density, and generality, respectively, absolute variation between local webs reconstructed by different techniques proved almost five, four, and two times as large as variation among webs from different parts of the world (Fig. 2 and Table S2).

Discussion

To understand how ecological communities vary in space and time, we need to understand how their members interact (1, 22). The present findings indicate that molecular analyses will significantly aid the discovery, identification, and quantification of interactions in natural communities. Overall, this integrative approach has the potential to radically transform the way in which we reconstruct and compare interaction webs across the globe.

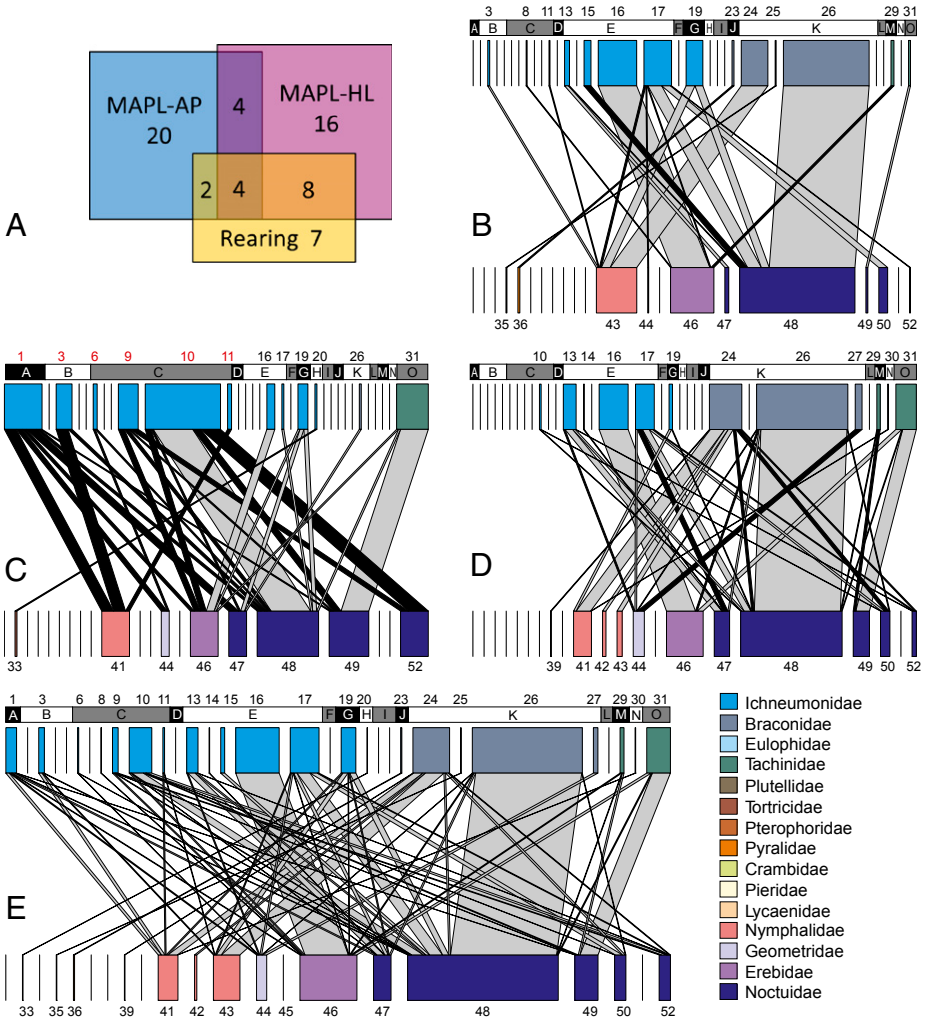

Fig. 1. Trophic links detected and visual representations of semiquantitative food webs reconstructed by three different techniques. (*A*) Venn diagram showing the number of interaction types (not frequencies) detected by each method, and the overlap among different methods. The sizes of the boxes correspond to the total number of trophic interaction types detected by each method. Numbers within boxes identify the set of links uniquely detected by each method, whereas numbers within overlapping sections show interactions detected by two or all three techniques. Method-specific food webs reconstructed by (*B*) rearing (redrawn from ref. 50), (*C*) MAPL-AP, and (*D*) MAPL-HL. In *E*, we show the web emerging from the combination of all three methods (rearing from ref. 50). In each web, blocks in the lower row represent hosts and blocks in the upper row represent parasitoids, with connecting lines identifying trophic links, and the width of the connector scaled to interaction frequency. In method-specific webs *B*–*D*, links detected uniquely by the method in question are marked in black. (Note that, strictly speaking, these representations of food web structure are semiquantitative, as the width of the boxes and the lines connecting them only reflect the number of individuals involved in each interaction, whereas no data on the specific abundances of hosts and parasitoids are provided. The total number of individuals on which each panel is based is detailed in Table S1) Across all webs, individual species are drawn in the same order. A, Pimplinae; B, Ichneumoninae; C, Cryptinae; D, Banchinae; E, Campopleginae; F, Cremastinae; G, Mesochorinae; H, Metopiinae; I, Euphorinae; J, Hormiinae; K, Microgastrinae; L, Eulophinae; M, Exoristinae; N, Dexiinae; O, Tachininae. Species for which trophic links were detected by the respective technique are identified by numbers, while species without links are shown as blocks without numbers. In web *C*, idiobiont parasitoids for which trophic links were detected are highlighted in red. Within families, subfamilies are shown in phylogenetic order, and within subfamilies, genera and species are shown in alphabetic order (as listed in Table S1).

As an illustration of the impact of the information used on the perception of web structure, we note that the method-specific variation in the structure of our target network was as large as or much larger than variation among five host–parasitoid webs from different parts of the world. As a web representing extremes in both latitude (high) and species richness (low), the web from Zackenberg exerts great leverage in any assessment of global patterns in food web structure. As a consequence, the method we select to depict it has the potential to change impressions of latitudinal patterns in interaction structure, or of the relation between the number of interacting species and the architecture of links between them (cf. refs. 15 and 16).

For interpreting the structure of our specific web, the patterns come with profound implications. Should previously proposed links between food web patterns and dynamics (18, 21, 22) stand the test of new methods, then the changes observed—from one of the least to perhaps the most highly connected web of antagonistic interactions observed in the world (compare with Fig. 2)—will change our inferences regarding its likely stability (21), and the likely resistance of its component species to extinctions (22). However, we do feel that the sensitivity of the Zackenberg web to methodological approach calls for a reevaluation of patterns across other webs as previously established by single techniques. To build a general understanding of food web structure versus dynamics, a larger number of webs should urgently be reexamined by multiple complementary techniques, including molecular tools.

At the level of species within the web, insights added by molecular techniques changed our impression of the role of different species, and types of species, on overall web architecture. Information derived from the gut contents of noncryptic adult predators shed light on trophic interactions involving their hidden larvae. Importantly, the connections revealed through such analysis had a major impact on overall food web structure, and accounted for a large proportion of taxa for which no prior links had been described (Fig. 1). In other systems, a molecular assessment of the gut contents of ubiquitous taxa may reveal the presence of more cryptic taxa forming part of the diet (40), the dietary choice of nocturnal predators which are hard to observe (39, 41), or differences in niche overlap among the same taxa in different environments (43). Quite remarkably, in the present study, two parasitoid sequences detected from within host larvae indicated the presence of species undiscovered before in the area (*SI Text*, section 5), despite the fact that the target fauna has been intensively sampled for 5 y (48, 50). These observations all expose how revealing molecular information sources may be in terms of exposing the smaller motifs (see also e.g., refs. 34 and 41–43) forming the basic building blocks of larger interaction networks (56–58).

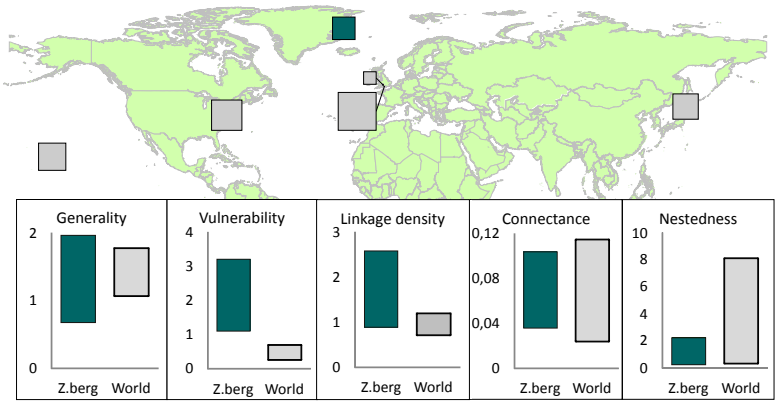

Fig. 2. Food web structure compared between methods and localities. The dark green box shows our study site (Zackenberg in Northeast Greenland) whereas gray boxes identify rearing-based host–parasitoid interaction webs reconstructed at other sites (see *Materials and Methods* for detailed references). The size of each box is proportional to the logarithm of matrix size (i.e., to the total sum of interactions in the respective quantitative network matrix). Below the map, we show variation in qualitative metrics describing interaction structure for method-specific networks reconstructed for Zackenberg (green boxes), and for rearing-based networks at other sites (excluding Zackenberg; gray boxes). For Zackenberg, the boxes identify the range of values reconstructed by different techniques (MAPL-AP, MAPL-HL, rearing, and their combination), whereas for the global comparison (World), the boxes show the range of values observed among food webs reconstructed at other sites. Individual metrics reflect the ratio of realized trophic links to all possible links within the web (connectance), the degree to which links from species with few links represent a subset of links from species with many links (nestedness), the average number of host species attacked by each parasitoid species (generality), the average number of parasitoid species attacking each host (vulnerability), and the average number of interactions per species (linkage density). [Note that for tractability, all metrics are here given in their qualitative form (according to ref. 86), but the relative difference between methods remains the same for quantitative estimates of generality, vulnerability, and linkage density. To calculate these metrics, all *Boloria* specimens were considered as one taxon.]

Our results indicate that different analytical approaches used to reveal predator–prey interactions are complementary. Among the three methods applied to describe our target web, the highest number of trophic links was detected through the sequencing of parasitoids within host larvae (MAPL-HL). Nonetheless, sole reliance on this method would have left nearly half of the trophic interactions undetected (for additional estimates, see *SI Text*, section 3). When combined, the two molecular methods (MAPL-AP and MAPL-HL) revealed most of the connections previously detected by rearing, and added many more. As the spreading out of interactions among both rare and common links—and the sum of weak interactions contributed by multiple rare species—have been suggested to have a strong stabilizing effect on overall food web dynamics (24, 25, 59, 60), resolving both types of links should be a key priority. The current results thus imply that the molecular methods are complementary, and that in future studies, traditional techniques such as rearing should (at the very least) be supported by these faster and less laborious molecular methods (but see *SI Text*, section 4 for further considerations). By determining the identity of the host (or prey) from the gut contents of the parasitoid (or predator) (30, 35, 61, 62), and/or by directly identifying the parasitoid DNA from the host (36), molecular approaches also circumvent problems associated with the detection of the feeding event (e.g., 63–65), or the maturation of the parasitoid (66, 67). Given the wide availability of PCR and the rapid advances in sequencing techniques, methods for detecting both hosts within parasitoids and parasitoids within hosts based on DNA barcodes is now an approach within reach for all ecologists (34, 43).

In conclusion, our results show how the information provided by molecular techniques can surpass that recovered by traditional techniques, but also that different types of molecular information are complementary, revealing different features in the emergent architecture of ecological interaction webs. By resolving more interactions than traditional techniques, by revealing interactions of species with a cryptic lifestyle, and by revising our impression of emergent food web structure, such combinations have the potential to revamp our impression of local food web structure, and how biotic interactions are patterned across the globe.

Materials and Methods

Given the practical problems associated with many methods for quantifying ecological interactions (e.g., refs. 63–65 and 68–71), a majority of antagonistic interaction networks described from terrestrial environments focus on a specific type of predator–prey interaction, i.e., host–parasitoid relations. Based on the specialized predation mode of parasitoids (discussed in the Introduction), trophic links involving such taxa have traditionally been quantified by the direct rearing of host individuals until they produce either an adult host or parasitoid (13, 72–75). The relative ease of constructing such webs has produced a substantial number of studies to date (16), yielding predictions in terms of global patterns in the structure (16), function, and dynamics (21, 22) of food webs. To exploit this comparative framework, we focused our dissection of interaction structure on this particular type of antagonistic association.

Study Area and Target Taxa. The number of possible links in an interaction web increases exponentially with the number of taxa involved, thereby calling for massive increases in sampling effort when describing progressively larger webs (16). We therefore focused our work on a High Arctic site with low species richness (48): the Zackenberg Valley (74°30′N, 20°30′W), located in Northeast Greenland National Park (76). The region is characterized by a High Arctic climate (77), with a total terrestrial fauna and flora of ∼500 and 163 species, respectively (48). The local lepidopteran community comprises 20 species representing 11 families, whereas their parasitoids include 30 species representing 3 hymenopteran families (Ichneumonidae with 19 species, Braconidae with 7 species, and Eulophidae with one species), and 1 dipteran family (Tachinidae, with three species) (48).

Sample Collection. Using a combination of semiquantitative methods (for details, see ref. 48), we collected 457 adult parasitoids and 1,195 lepidopteran larvae from June to August in 2011 and 2012. In brief, the primary method used was visual search, with additional use of live-trapping pitfall traps and sweep netting. To avoid contamination, we collected all insects individually and stored them in separate tubes filled with 99.5% ethanol. The samples were identified in the field by R.K., G.V., and T.R. (48). Uncertain cases were examined with a microscope and a DNA barcode was generated to verify their identification. For two species of butterflies in the region [*Boloria chariclea* (Schneider, 1794) and *Boloria polaris* (Boisduval, 1828)], the larvae are currently undescribed and can therefore not be identified by morphological characters. To resolve species-specific trophic interactions involving *Boloria*, we identified 74 larvae via DNA barcoding (47). The rest (n = 17 *Boloria* larvae) were treated as a compound taxon.

DNA Barcode Library Creation. A reference library for the barcode region of the mitochondrial cytochrome c oxidase 1 (CO1) gene was generated for the target groups, adhering to standard protocols at the Canadian Centre for DNA Barcoding (CCDB) [refs. 78 and 79 and CCDB protocols by Ivanova and Grainger (http://ccdb.ca/resources.php)]. Data on voucher specimens, including images and DNA barcode sequences, have been deposited in BOLD Systems (49) and DNA barcode sequences have deposited in both BOLD (dx.doi.org/10.5883/DS-GRBARDOI) and GenBank (accession nos. KF604304–KF604627).

Primer Design. For detecting host DNA from within adult parasitoid (MAPL-AP), we adopted the approach developed by Rougerie et al. (36). To detect potentially degraded and/or low-yield host or parasitoid DNA (80), we designed unique primers to amplify a short but variable region of the CO1 gene. As a complementary approach, we wanted to amplify and sequence parasitoids from within the larval hosts, and therefore designed another method (MAPL-HL) based on unique primers. Using an alignment of full-length (658-bp) barcodes from all available host and parasitoid species in the study area (listed in Table S1), we designed four primer sets targeting a 148-bp hypervariable region, that enabled the identification of all Lepidoptera and parasitoid species at Zackenberg. Each primer set was chosen to include at least one primer binding to all of our potential host species, but to none of the potential parasitoid species (or vice versa; for details and exact primer sequences, see *SI Text*, section 6 and Table S1). All primers were tailed with a modified M13F or M13R sequence (81), which was subsequently used as the sequencing primer for all PCR products.

DNA Extraction, PCR, and Sequencing. For both MAPL-AP and MAPL-HL the optimal tissue sampling was tested (details in *SI Text*, section 7), resulting in the head and abdomen as the best choices for MAPL-AP, and the whole larva for MAPL-HL. DNA was extracted using the glass-fiber protocol of Ivanova et al. (78). Before amplification, the purified DNA was diluted to 1:10 or 1:100 for Lepidoptera tissue samples (depending on the amount of tissue originally used) and to 1:10 for samples of parasitoid tissue. PCR conditions followed standard CCDB protocols (79), with the exception of thermocycling, which depended on the primers used (details in *SI Text*, section 6). PCR products were Sanger (82) sequenced without cloning and sequence editing followed standard CCDB protocols (79). Special care was taken to avoid contamination at all steps of the protocol, always using sterile equipment, blank controls both in extraction and amplification, and treating pre- and post-PCR products in different laboratories. These MAPL-AP and MAPL-HL sequences have been deposited in BOLD (dx.doi.org/10.5883/DS-GRMAPDOI) and in GenBank (accession nos. KF448119–KF448508 and KF646825–KF646829).

Identification of Food Web Nodes and Link Structure. The assignment of species to sequences was conducted with the BOLD identification engine (49), searching all barcode records available in BOLD. We used a lower threshold of 98% identity to assign a sequence record to a species. Two cases were encountered where the match to all reference sequences from species known from the area were lower than 98%, suggesting species not found before in the area and/or potential cryptic variation (details in *SI Text*, section 5).

Food webs based on the host–parasitoid linkages revealed by MAPL-AP, MAPL-HL, and rearing were drawn in R (83) using the bipartite package (17). Qualitative metrics of connectance, generality, vulnerability, and linkage density were calculated by hand (Fig. 2 and Table S2), whereas quantitative metrics of the above-mentioned metrics (used for comparison) were calculated in bipartite (17). The data for the rearing-based food web of the study area was adapted from ref. 50.

Variation in Network Structure: Methods versus Global Patterns. To gauge relative variation in network structure against a clear-cut reference point, we extracted data on all interaction networks compiled for a recent meta-analysis on global patterns in the structure of antagonistic interaction networks (16). As the biology of the host may influence the specificity of its interactions with other species (46, 84, 85), we specifically targeted fully quantified webs consisting of free-feeding insects and their parasitoids. A set of five such webs was identified, all reconstructed by traditional rearing. These webs were derived from the continental United States (52), Hawaii (10), the United Kingdom (53, 54), and Japan (55). For ref. 53, the specific structure of the web was extracted from explicit diagrams and an appendix in the primary publication, whereas for all other webs, specific information was obtained from the relevant author. Because most studies consisted of data collected over multiple sites or years, we partitioned the data into year- and site-specific subwebs matching the spatial and temporal scale of our own study, calculated metrics for individual webs ($n = 8$ for ref. 53, $n = 1$ for ref. 54, $n = 1$ for ref. 10), $n = 4$ for ref. 52, and $n = 2$ for ref. 55), then used a single mean as derived across subwebs as an individual observation. In cases where the original web included multiple guilds of herbivores, we extracted data on trophic interactions between the free-feeders and their parasitoids only, again to match the scope of our Arctic web. Specific figures behind the general patterns summarized in Fig. 2 are given in Table S2.

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